



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 31/00, 31/16, 33/02, 21/00	A1	(11) International Publication Number: WO 98/20337 (43) International Publication Date: 14 May 1998 (14.05.98)
(21) International Application Number: PCT/US97/20097 (22) International Filing Date: 4 November 1997 (04.11.97) (30) Priority Data: 08/744,111 5 November 1996 (05.11.96) US 08/925,849 8 September 1997 (08.09.97) US (71) Applicant: BIODETECT CORPORATION [US/US]; Stabile Center, 2 Wellman Avenue, Nashua, NH 03060 (US). (72) Inventors: WALLACH, Donald, F., H.; 38, route de Malagnou, CH-1208 Geneva (CH). NOVIKOV, Alexander; 22 Hiram Road, Framingham, MA 01701 (US). (74) Agents: LOREN, Ralph, A. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHODS AND DEVICES FOR DETECTING SPOILAGE IN FOOD PRODUCTS (57) Abstract The present invention pertains to methods and devices for detecting spoilage of a food product. The method involves placing a spoilage indicator device including a barrier sheet in fluid contact with a food product. The method also involves allowing any reactant molecule of a predetermined size produced in the food product by spoilage to traverse the barrier sheet to contact a carrier of the device and to react with a pH indicator material therein. The method further provides observing the spoilage indicator to determine whether the detectable change has occurred in the pH indicator material. The detectable change indicates a build-up of the reactant molecule in the food product and therefore is indicative of spoilage.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

METHODS AND DEVICES FOR DETECTING SPOILAGE IN FOOD PRODUCTS

Background of the Invention

5

The field of this invention concerns the determination of food freshness, and, in particular, methods and devices for the detection of spoilage in food products. The spoilage and souring of perishable foods with time is an on-going problem for the consumer and food product provider alike. Deterioration in freshness is in large part due to the growth of microbes such as bacteria, yeasts, and fungi. To derive energy for their growth, these microbes break down food carbohydrates, proteins and fats. The breakdown process produces a variety of low molecular weight molecules capable of producing hydrogen ions that affect pH. Such molecules include carboxylic acids (e.g., lactic and acetic acids), aldehydes, nitrogen containing molecules including ammonia, trimethylamine and small diamines, and some sulfur compounds. For example, over time, microbes in milk and dairy products produce an increased amount of lactic acid and lactic acid derivatives resulting in sour and odorous milk, respectively. The generation of lactic acid and its derivatives creates a more acidic environment with a lower pH. (See, e.g., Gyosheva, B. H., (1982) "Compounds forming the aroma complex of Bulgarian sour milk" *Milchwissenschaft* 37, 267-289).

20

At present, there are no inexpensive, simple and accurate measurement devices for detecting food spoilage at a consumer level. Spoilage has conventionally been monitored by standard bacteriological and chemical laboratory methods, while certain more esoteric assays have been discussed in the literature to improve speed or cost of detection. For example, electrochemical assays involving gamma-irradiation immobilization of lactate oxidase in poly (vinyl alcohol) on platinized graphite electrodes have been proposed for lactate detection in dairy products (See Hajizadeh, K., *et al.* (1991) "Immobilization of lactate oxidase in a poly(vinyl alcohol) matrix on platinized graphite electrodes by chemical cross-linking with isocyanate" *Talanta* 38, 37-47). Other approaches to food spoilage monitoring include, for example, using non-membrane disposable oxygen electrode systems to detect milk spoilage from aerobic bacteria (See Bell, C. Ackland, *et al.* (1995) "Disposable oxygen electrode system without membranes applied to the detection of ultrahigh-temperature milk spoilage" *Netherlands Milk and Dairy Journal* 49, 139-149); and modified Orion ammonia electrodes for the trimethylamine detection in fish (See Chang, G.W., *et al.* (1976) "Trimethylamine-specific electrode for fish quality control"

35

Journal of Food Science 41 723-724). However, these techniques are not practical at a consumer level and smell, color and taste have remained the predominate methods used by consumers to detect spoilage.

5 Recently, there have been attempts at finding a method consumers can use to determine whether food products are contaminated by specific toxins. For example, U.S. Patent No. 5,306,466 by Goldsmith discloses a packaging method which includes a bar code design consisting of labeled antibodies bound to toxins. The bar code design is placed in contact with the food product, for example by printing it on a membrane in the packaging
10 itself, and a competitive antibody-antigen reaction is used to detect spoilage. The antibodies on the membrane, which react with the specific class of toxins accumulating in the food product, are released from the membrane, thereby destroying the design and providing a visual indication of toxin presence. However, this type of indicator is expensive because of the antibody cost, may not be safe to consumers, and has limited
15 applicability.

 Similarly, electrode systems and electrochemical or competitive assay techniques, do not solve the consumer problem. These techniques often involve relatively lengthy or complex procedures, and may have limited applicability. Accordingly, there
20 exists a need for relatively rapid, efficient, accurate, inexpensive and simple methods and devices for indicating spoilage in a variety of food products. Such methods and devices would simplify spoilage indication techniques while maximizing their accuracy, efficacy and applicability through the selection of indicators, buffers and barriers.

25 To give the consumer an indication of when spoilage may occur, "expiration dates" are currently stamped on containers of milk, juice and some other foods. Such "date stamps" suggest that a food sample under conditions of purchase will not "turn" prior to the expiration date. This is statistical, not specific, information, and these "date stamps" have no meaning after the container is opened.

30 Recently attention has focused on Time-Temperature indicators (TTI) to monitor the quality of refrigerated, controlled-atmosphere-packaged and deep-frozen foods. (See Labuzda, T.P. and Bin Fu, 1995, "Use of time/temperature indicators, predictive microbiology, and related technologies for assessing the extent and impact of temperaure
35 abuse on meat and poultry products". *J. Food Safety* 15, 201-227; and J.H. Wells and R.P. Singh, 1992, "The application of time-temperature technology to food quality monitoring and perishable inventory management". in Thorne, S. ed. *Mathematical modelling of food*

- 3 -

processing operations. p. 271-344, Elsevier Applied Sciences, London). Similarly, patents by T.J. Jalinski describe methods of monitoring a possible unfavorable temperature history of refrigerated foods. (Time temperature indicator with distinct end point; label for food packaging indicating refrigerated storage, U.S. Patent 5,182,212 and time temperature indicator with distinct end point; dual system of active reagent, substrate and pH-sensitive dye for monitoring shelf-life of cooked packaged food, room temperature or refrigerated storage. U.S. Patent No. 5,085,802, Commercial TTI include the Life Line Monitor, the I-Point Time/Temperature Monitor, and the 3M Monitor Mark. The TTI approach is expensive and complicated at the retail consumer level and implementation will require much consumer education.

Accordingly, an object of the invention is to provide a method of indicating food spoilage which is simple, accurate and inexpensive.

Another object of the invention is to provide a food spoilage indicator device for placing in fluid contact with a food product.

These and other objects and features of the invention will be apparent from the following description and the drawings.

Summary of the Invention

The present invention provides a method of detecting spoilage in food products using the described spoilage indicator device. The spoilage indicator device has a barrier sheet which is permeable to uncharged molecules less than 200 daltons in size and is substantially impermeable to larger uncharged and charged molecules. The barrier sheet is disposed in contact with the food product. The spoilage indicator device also includes a carrier which has been impregnated with a pH indicator material and buffer material. The pH indicator material is capable of producing a visually detectable change upon reacting with a reactant molecule produced in the food product when the food product is undergoing spoilage. The first planar surface of the carrier is separated from fluid contact with the food product by the barrier sheet. The spoilage indicator device also includes a transparent or translucent outer layer disposed on a second planar surface of the carrier. This outer layer protects the pH indicator material and buffer material impregnated within the carrier from contamination associated with the food packaging.

The method of the present invention allows the desired reactant molecules produced by food product spoilage to traverse the barrier sheet separating the food product from the carrier and react with the pH indicator material in the carrier. The reactant molecule can be carried in a liquid phase, gas phase, acid or base and the pH indicator of the present invention is capable of reacting with the reactant molecule in each of these situations. The detectable change caused by the reaction of the reactant molecule with the pH indicator is a visible change in color which indicates a build-up of the reactant molecule in the food product which provides an indication of spoilage.

The device of the present invention includes a barrier sheet composed of the hydrophobic materials polyethylene or polyvinyl chloride with a thickness in the range of 10 to 15 microns. The carrier layer of the spoilage indicator device consists of a paper and has a thickness in the range of 15 to 20 microns. The pH indicator material of the spoilage indicator device is selected from the group consisting of phenol red, cresol red and *m*-cresol purple. The buffer material of the spoilage indicator device should be effective within the pH 8-10 range and preferred buffer systems consist of amino acids with the most preferred buffer systems consisting of lysine or glycine. The transparent (translucent) outer layer of the spoilage indicator device has a thickness in the range of 5 microns to several millimeters and may be composed of various materials depending upon the position of the device. In situations where the outer layer of the spoilage indicator device is in contact with the ambient atmosphere, the outer layer consists of a gas-impermeable material selected from the group consisting of polyester, cellulose acetate, polyolefin, polypropylene polystyrene, polyvinylidene chloride and glass. In situations (e.g., in "clamshell packages") where the outer layer of the spoilage indicator device is in fluid contact with the food product, the outer layer consists of the gas-permeable materials polyvinyl chloride or polyethylene.

As used herein, the following terms are to be understood in light of the following definitions:

The term "fluid" refers to liquid or gaseous states;

The term "barrier" means a device or portion of a device which provides a physical separation between one location and another or between the device and the product;

The term "reactant molecule" means any molecule produced in a food product causing spoilage, or a product of a secondary reaction of a molecule produced in the food product causing spoilage, which can react with the selected pH indicator in the device of the invention;

- 5 -

The term "indicator" means any material which can react with the reactant molecule to produce a detectable, e.g., visual change, either through a direct or intermediate reaction;

5 The term "spoilage" means any reduction in freshness of a food product making it less palatable or dangerous for consumption by the animal, e.g., human, that would normally eat the food product; and

The term "carrier" means any material which entraps or holds an indicator; e.g., paper, other fibrous or cellulosic materials and the like.

10 The term "buffer" means any material which maintains pH reasonably constant when additional acid and base is added.

Other features and aspects of the invention will be apparent from the detailed description and the drawings.

15 Brief Description of the Drawings

FIG 1 is an enlarged cut-away side view of the spoilage indicator device, showing a barrier sheet disposed on a first surface of the carrier containing a pH indicator material and buffer material; and

20

FIG 2 is a cut away of another embodiment of the spoilage indicator device, wherein the device has a sealed outer surface.

Detailed Description

25

The present invention features methods and devices for providing a visibly detectable indication of food spoilage. The spoilage indicator device contains a barrier sheet which allows passage of uncharged reactant molecules of a predetermined size but segregates out charged and larger uncharged molecules. The barrier sheet is in contact with
30 the food product and is contiguous to the first planar surface of the carrier which has been impregnated with a pH indicator material and buffer material. The barrier sheet protects the carrier from potential contamination. The pH indicator material provides a visually detectable change, a color change, upon reaction with the reactant molecules which indicates spoilage. The present invention also contains an outer layer that separates the
35 second planar surface of a carrier from the ambient atmosphere or the fluid phase of the food product. The present invention is based on the further recognition that such a device can be placed in a food product package without contaminating the food.

Reactant molecules produced by food products undergoing spoilage exist in liquid and gaseous forms and impact the pH of the fluid phase of the food product.

Reactive molecules produced by food spoilage include acids, bases, aldehydes, sulfur compounds, and their derivatives. Particular examples of reactant molecules resulting from spoilage include carboxylic acids, such as acetic or lactic acids; acid derivatives such as acetylaldehyde, basic molecules containing nitrogen such as ammonia, and amines (e.g., trimethylamine and small diamines having a molecular weight of less than about 200 daltons) and sulfur containing molecules such as hydrogen sulfide. The barrier sheet of the present invention is selected to allow the desired reactant molecule to pass through while retaining the pH indicator and buffer materials and excluding larger, unwanted molecules.

Thus, the desired reactant molecules produced by food product spoilage are allowed to traverse the barrier sheet separating the food product from the carrier and react with the pH indicator material in the carrier. The detectable change caused by the reaction of the reactant molecule with the pH indicator is a visible change in color which indicates a build-up of the reactant molecule in the food product which provides an indication of spoilage.

In the present invention, suitable barrier materials must be permeable only to uncharged molecules less than 200 daltons in size and substantially impermeable to larger uncharged molecules. In addition, the barrier material must be chemically inert and non-contaminating to the food product. Preferred barrier materials include, but are not limited to, low- or medium- density polyethylenes, polyvinyl chlorides and other water resistant or hydrophobic materials meeting Food and Drug Administration standards for food wrappings. Typically, the barrier sheet has a thickness in a range of 10 to 15 microns. Preferably, the barrier sheet extends beyond the contours of the carrier to form a totally sealed package. This type of device is particularly useful if liquids are to be use since it will protect the carrier against contamination by splashing.

Various carriers for entrapping an indictor material are known to those of ordinary skill in the art. Examples of such carriers include paper (e.g., untreated cellulose), polyamides, cellulose acetate, gels, foams, glass fibers and resins such as ion-exchange resins. A transparent polyester layer can be adhered to the carrier to stiffen it and make it easier to cut and handle. This layer can also be used as the outer layer.

In the present invention, the first planar surface of the carrier is contiguous to the barrier sheet and has been impregnated with a pH indicator material and buffer material. The carrier material must be capable of uniformly absorbing the pH indicator material and buffer material and must also allow the reactant molecules access to the pH indicator. As the carrier material will be observed visually and/or photometrically, the material must allow for a good contrast between the acid and basic colors of the absorbed pH indicator. In addition, the carrier material should be non-reactive with the pH indicator and buffer and possess minimal fixed charge and little ash content. As a result, porous, inert materials are preferred carrier materials. The carrier generally has a thickness in the range of 15 to 25 microns. The preferred carrier material is a paper, specifically, low-lint, high density, facial tissue-type paper. In addition, other similar personal care-type "tissues" may also prove equally satisfactory.

Impregnated within the carrier, the preferred pH indicator material has a pK close to the pK range of the food product and produces a substantial color change in response to very small changes in hydrogen concentration (e.g. less than 10^{-7} M $[H^+]$). In addition, the preferred pH indicator material is sensitive enough to produce detectable changes when the reactant molecules are in a concentration of approximately 0.01% by volume in the fluid surrounding the food product. Specific examples of preferred indicator materials, include but are not limited to, phenol red (phenolsulfonephthalein), *m*-cresol purple (*m*-cresolsulfone-phthalein), cresol red (*o*-cresolsulfonephthalein) and mixtures thereof. All the preferred indicators are characterized by a strong change in color and an increase in color intensity moving from pH 7-7.5 to pH greater than 8.

Examples of pH indicator materials, as well as the pH ranges within which they are effective and what color changes occur, are shown in Table 1. The preferred indicators are in bold.

Table 1
Indicator systems

Indicator	pK	Acid* color	Basic* color	λ Max acidic/basic	pH range
Neutral red (3-amino acid-6-d-methylamino-2-methylphen-azinium chloride)		red	brown-yellow		6.8-8.0
Phenol red (phenolsulfonephthalein)	7.9	yellow	red	~420/~555	6.8-8.4

<i>m</i> -Cresol purple (<i>m</i> -cresolsulfone-phthalein)	8.3	yellow	violet	~420/~555	7.6-9.2
Cresol red (<i>o</i> -cresolsulfonephthalein)	8.46	yellow	purple	~420/~555	7.2-8.8
Thymol blue (thymosulfonephthalein)	9.2	yellow	blue	~378/~590	8.0-9.6

* The shades of the acidic and basic colors depend on the buffer systems

As pH indicators with a pK greater than 7.0 do not exhibit stable basic colors unless buffered, the use of appropriate buffer/indicator combinations is essential to maintain stable initial colors. In order to achieve stable initial colors, buffer concentrations of 0.01 to 0.05 M or buffer/indicator molar ratios of 20 to 200 (typically 50) are required. At such buffer/indicator ratios, the response of the spoilage indicator device to acid or basic volatiles is predominately that of the buffer. Raising the indicator concentration enough to significantly affect the buffer ratio will obscure color change and lowering buffer concentration significantly decreases initial color stability. The preferred buffers for general use include lysine and glycine. Additional amino acid derivatives which may also be of use as a buffer system include; N,N-(Bis-2-hydroxyethyl)glycine; 1,5,(4)dimethylimidazole; 2-amino-2-methyl-1,3,-propanediol; 2-amino-2-ethyl-1,3,propanediol, diethanolamine; ethanolamine,2-(cyclohexylamino)ethane sulfonic acid and 2-amino-2-methyl-1-propano. Borate buffers also appear equally suitable in principle to lysine and glycine but are not as preferred for food applications. In addition, carbonate buffers are suitable for use in carbonated beverages.

Examples of several buffer systems and their pK values are shown in Table 2. The generally preferred buffers are indicated in bold.

5

Table 2
Buffer systems.

Buffer system	pK	
Tris (hydroxymethyl)aminomethane	8.08	
Lysine	9.06	(pK₂)
	10.53	(pK₃)
Borax	9.14	(pK ₁)
Glycine	9.78	(pK₂)
Bicarbonate/carbonate	10.25	(pK ₂)
Phosphoric acid	6.82	(pK ₂)
	12.39	(pK ₃)
Arginine	8.99	(pK ₂)
	12.48	(pK ₃)

The spoilage indicator device of the present invention also includes an outer layer which is transparent, translucent and/or has at least a translucent or transparent portion through which an underlying layer, such as the carrier, can be observed visually and/or photometrically. The outer layer is normally disposed directly on the second planar surface of the carrier material. Depending on the application of the device, the outer layer can have a thickness ranging from 0.07 to 2.5 mm. For applications in which both the barrier sheet and the outer layer of the spoilage indicator device are in fluid contact with the food product, both layers are composed of a material permeable to reactant molecules in order to increase the diffusion area of the device. In these situations, the preferred materials for the outer layer include, but are not limited to, polyvinylchloride and polyethylene. For applications in which the outer layer of the spoilage indicator device is in contact with the ambient atmosphere, a gas-impermeable, food-packaging grade plastic can be used for the outer layer. In this situation, the preferred materials for the outer layer include, but are not limited to, polyester, cellulose acetate and polyvinylidene chloride (Saran Wrap).

The following Figures help illustrate the devices and methods of the invention. FIG. 1 shows a cut-away view of an embodiment of the spoilage indicator device 110. Device 110 has a carrier 114 with a first surface 115 directed toward food product 126. Carrier 114 has a pH indicator material and buffer material 120 entrapped within it. First surface 115 of carrier 114 is separated from contact with food product 126 and its surrounding juices 128 by a barrier 118. Spoilage indicator device 110 as shown also includes a transparent or translucent outer layer 116 disposed on a second surface 117 of carrier 114. Such an outer layer 116 protects the pH indicator material and buffer material 120 entrapped within the carrier 114 from contamination associated with the food packaging.

The barrier layer 118 is disposed directly on the first surface 115 of carrier 114 and may continue beyond the contours of the carrier 114 to form the wrap or food packaging, thereby eliminating additional layers between the carrier 114 and the food. Similarly, outer layer 116 may continue beyond the contours of carrier 114 in the form of a tape or label.

As shown in Fig. 2, the device may have a barrier sheet 218 wrapped around carrier 214, covering both first and second carrier surfaces 215 and 217. Preferably, barrier sheet 218 also extends beyond the contours of the carrier 214 to form a totally sealed package. This type of device is particularly useful if liquids are to be use since it will protect against contamination by splashing.

FIG. 2 further shows the outer layer 216 is sealed with a food-package grade, water- and cold-insensitive tape 219. The tape 219 adheres to the outer layer 216 and to the wrap or food packaging layer 212 if used separately. It is advantageous that tape 219 is provided with a transparent or translucent window 221 to observe the detectable changes of the pH indicator material 220. Alternatively, tape 219 itself is transparent or sufficiently translucent to allow an observer to view a detectable change in the pH indicator material 220 through the tape 219.

In a preferred embodiment of the invention, the spoilage indicator device includes cresol red as the pH indicator and lysine as the buffer impregnated within a paper carrier for detecting lactic acid and other reactant molecules produced by the spoilage of milk. In this embodiment, the carrier's first planar surface is separated from fluid contact with the food product by a barrier sheet composed of polyvinyl chloride and an outer layer composed of Saran Wrap (see Example 7).

In another preferred embodiment of the invention, the spoilage indicator device includes phenol red as the pH indicator and lysine as the buffer impregnated within a paper carrier for detecting acetic acid and other reactant molecules produced by the spoilage of citrus juices. In this embodiment, the carrier is separated from fluid contact with the food product by a barrier sheet composed of polyethylene film and an outer layer composed of Saran Wrap (see Example 4). In a most preferred embodiment, the spoilage indicator device also includes a "window" or a transparent or translucent layer which allows visualization of the pH indicator from outside the packaging. This window could be a cellulose acetate, polyvinylidene chloride, polyester or glass outer layer disposed on a second planar surface of the carrier.

In use, the spoilage indicator device of the present invention can be placed such that the pH indicator is in fluid contact with the food product and/or its surrounding fluid phase. As discussed above, fluid contact with the food product includes either liquid or gaseous contact. The pH indicator device can be placed in a variety of ways known to those skilled in the art so long as its placement allows reactant spoilage molecules to contact and to traverse a barrier sheet. Therefore, the spoilage indicator device may be placed in a bottle cap or other part of a liquid containing package without fear of contamination.

The alternatives described above are not intended to limit the practice of the invention. The exemplary indicator materials, carriers, barrier sheets and outer layers discussed above are presented for illustrative purposes only and are not intended to limit the invention. The following non-limiting Examples further illustrate the efficacy of the invention.

Example 1

In this example, the response of the spoilage indicator to different concentrations of sodium bicarbonate in 0.1 M phosphate buffer were tested. The spoilage indicator device used PVC as an inner layer and 0.01% cresol red plus 0.01% *m*-cresol purple in 0.05M Lysine buffer, pH 9.05, as indicator. Detection was carried out in the gas phase at room temperature. The color of the sensor changes from purple, P, to colorless 0, with increasing bicarbonate

10

		Bicarbonate concentration (%/)					
		0	0.005	0.01	0.02	0.05	0.1
Color							
30 min	P	P	P↓	P↓↓	0	0	
120 min	P	P↓	P↓	P↓↓	0	0	

The CO₂ concentration is related to pH by the following formula:

$$\log[\text{CO}_2] = \text{pK}' - \text{pH} + \log [\text{HCO}_3]$$

15

where $\text{pK}' = 6.35$ and pH is set at 5.60. A bicarbonate concentration of 0.05% corresponds to 0.0006 M. At 0.0006 M bicarbonate, $\log[\text{CO}_2] = 0.75 + \log [0.0006] = 0.75 - 3.22 = -2.47$. The concentration of CO₂ is therefore 0.0034 M.

20

This example shows that the spoilage indicator device is sensitive to bicarbonate concentration.

Example 2

In this example, the response time of the spoilage indicator device to the permeation of several small organic acids was monitored. The barrier sheet was composed of PVC cling wrap (thickness 11.4 microns), the carrier was composed of EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was 10 microliters of 0.02% cresol red, the buffer material used was 0.01M lysine (pH 9.45), and the outer layer was composed of polyvinylidene chloride (Saran) cling wrap (thickness ~ 7 microns). The spoilage indicator device was located 2.5 cm above the test liquid for gas phase detection of the reactant molecules. The indicator material, cresol red, is violet (V) at a pH > 9 and yellow (Y) at a pH < 8.

The results show that the spoilage indicator device is much more sensitive to acetic, propionic and butyric acids than lactic acid. Thus, 0.1 M lactic acid (0.9%) produces a detectable change only after 18 hours in this system, whereas 0.001 M butyric acid (0.009%, i.e. at 1/100th the concentration) reacts within 3 hours in this system. Thus, propionic and butyric acids are more easily detected, although they are generally considered lesser end products of food spoilage. Within the summary table below, "↓" indicates a decrease in color and significant changes of color are indicated in bold.

		Time (hrs)			
Color at		0	3	18	44
Water		V	V	V	V
<u>Acetic acid</u>	0.01 M	V	YV	Y	Y
<u>Propionic acid</u>	0.001 M	V	V	↓V	Y
<u>Butyric acid</u>	0.001 M	V	↓V	VY	Y
<u>Lactic acid</u>	0.1 M	V	V	↓V	VY

Example 3

In this example, the response time of the spoilage indicator device to the spoilage of different volumes of aged orange juice was assessed. The barrier sheet of the device was composed of PVC, the carrier material used was EX-L Kimwipe tissue paper (thickness ~ 18 microns), the indicator material used was 0.01% cresol red plus 0.01% *m*-cresol purple, the buffer material used was 0.05M lysine (pH 9.05) and the outer layer was composed of Saran Wrap. The device was placed above the orange juice for gas phase detection and was monitored at room temperature.

The results indicate that the device is more sensitive to a greater volume of food product with a color change visible after 15 minutes with 13ml of juice and after 30 minutes with 2ml of juice. The color of the indicator material was noted to change from purple (P) to colorless (O) to yellow (Y) as spoilage progressed and acidity increased.

		Time (min)				
Color at		0	5	15	30	60
Volume = 13 ml 2 ml		P	P	P↓	O	Y
		P	P	P	P↓	O

Example 4

In this example, the response of the spoilage indicator device in direct contact with citrus juices was monitored at different temperatures. The barrier sheet was composed of Cling Wrap polyethylene film, the carrier material used was EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was 0.01% phenol red, the buffer material used was 0.05M Lysine/NaOH (pH 8.6) and the outer layer was composed of Saran Wrap. The device was initially positioned above the citrus juices and then the vials were turned upside down to put the device in direct contact with the food product. The experiment was conducted with both fresh and aged juices at both room temperature and 10°C.

As expected, the most rapid color change was detected with the aged juice at room temperature. In addition, the device appears to be more sensitive to spoiling grapefruit juice than spoiling orange juice. The color of the indicator material was noted to change from purple (P) to yellow (Y) as spoilage progressed and acidity increased. Significant changes of color are indicated in bold. This example illustrates that the spoilage indicator device can differentiate between food and aged orange juice.

	RT (min)				10°C (min)			
	0	30	60	120	0	120	480	1080
Water	P	P	P	P	P	P	P	P
Orange J.								
Fresh	P	P	P	P↓	P	P	P	P↓
Aged	P	P↓	Y	Y	P	P↓	Y	Y
Grapefruit J.								
Fresh	P	P	P↓	P↓	P	P	P	P↓
Aged	P	Y	Y	Y	P	Y	Y	Y

Example 5

In this example, the response time of the spoilage indicator device to the spoilage of meat, chicken, fish and juices was assessed. The barrier sheet was composed of PVC film, the carrier material used was EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was 0.01% cresol red plus 0.01% *m*-Cresol purple, the buffer material used was 0.05 M lysine (pH 9.16) and the outer layer was composed of Saran Wrap. The device was placed above the food product at room temperature for gas phase detection of the reactant molecules.

The color of the indicator material was noted to change from bluish purple (P) to yellow (Y) as spoilage progressed and acidity increased. The device was noted to be more sensitive to the spoiling aged juices rather than the meats. Significant changes of color are indicated in bold.

15

		Time (min)						
Color at		0	5	15	30	60	180	360
Water		P	P	P	P	P	P	P
Hamburger	fresh	P	P	P	P	P	P	P
	aged	P	P	P	PY	PY	Y	Y
Chicken	fresh	P	P	P	P	P	P	P
	aged	P	P	P	PY	PY	Y	Y
Trout	fresh	P	P	P	P	P	P	P
	aged	P	P	P	PY	PY	Y	Y
Orange J.	fresh	P	P	P	P	P	P	P
	aged	P	P	P	PY	Y	Y	Y
Grpfr. J.	fresh	P	P	P	P	P	P	PY
	aged	P	PY	PY	Y	Y	Y	Y

Example 6

In this example, the response time of the spoilage indicator device to the spoilage of meat, chicken, fish, milk and juices at different temperatures was assessed. The barrier sheet was composed of Cling-Wrap polyethylene film, the carrier material used was EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was 0.01% cresol red plus 0.01% *m*-cresol purple, the buffer material used was 0.05 M lysine (pH 9.4) and the outer layer was composed of Saran Wrap. The device was positioned above the food product for gas phase detection of the reactant molecules.

The color of the indicator material was noted to change from purple (P) to yellow (Y) as spoilage progressed and acidity increased. Significant changes of color are indicated in bold. The results indicate that the device is most sensitive to spoiling aged grapefruit juice and equally sensitive to spoiling aged meats, milk and orange juice.

		Time (hours)		
		Room temperature		10°C
Color at		0	2	18
Water		P	P	P
Hamburger	fresh	P	P	P
	aged	P	P↓↓	Y
Chicken	fresh	P	P	P
	aged	P	P↓↓	Y
Fish	fresh	P	P	P
	aged	P	P↓↓	Y
Orange J.	fresh	P	P	P↓
	aged	P	P↓↓	Y
Grpfr. J.	fresh	P	P↓	P↓↓
	aged	P	Y	Y
Milk, whole	fresh	P	P	P
	aged	P	P↓↓	Y
Milk, skim.	fresh	P	P	P↓
	aged	P	P↓↓	Y

Example 7

In this example, the response time of the spoilage indicator device to the spoilage of fresh and aged milk at both room temperature and 10°C was assessed. The barrier sheet was composed of PVC film, the carrier material used was EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was cresol red 0.02%, the buffer material used was 0.05 M lysine (pH 9.4) and the outer layer was composed of Saran Wrap.

The results indicate that a color change can be detected after 2 hours and that the lower the pH the more pronounced the color change. The color of the indicator material was noted to change from purple (P) to colorless (0) to yellow (Y) as spoilage and acidity increased. Significant changes of color are indicated in bold.

Color at	RT (hrs)		10°C (hrs)			
	0	2	18	40	72	168
Whole milk						
Fresh	P	P	P	P	P	P
Aged, pH 5.98	P	P↓↓	Y	Y	Y	Y
Aged, pH 6.24	P	P↓	P↓	Y	Y	Y
Skim milk						
Fresh	P	P	P	P	P	P
Aged, pH 6.45	P	P↓↓	Y	Y	Y	Y
Aged, pH 6.57	P	P↓	0	Y	Y	Y

Example 8

In this example, the response time of the spoilage indicator device to the spoilage of fresh and aged milk was monitored. The barrier sheet was composed of PVC, the carrier material used was EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was cresol red 0.02%, the buffer material used was 0.05 M lysine (pH 9.4) and the outer layer was composed of Saran Wrap. The device was placed above the milk at room temperature for gas phase detection of the reactant molecules.

A color change was detected for aged skim and whole milk after 30 minutes and after 3 hours for fresh skim milk. No color change was detected for fresh whole milk within 3 hours. The color of the indicator material was noted to change from purple (P) to yellow (Y) as spoilage progressed and acidity increased. Significant changes of color are indicated in bold.

		Time (min)				
Color at		0	10	30	60	180
Water		P	P	P	P	P
Milk, whole, fresh pH 6.6		P	P	P	P	P
Milk, whole pH 6.2	aged	P	P	PY	Y	Y
Milk, skim, fresh pH 6.7		P	P	P	P	PY
Milk, skim, pH 6.3	aged	P	P	PY	Y	Y

Example 9

In this example, the response time of the spoilage indicator device to the spoilage of meat, chicken and fish at both room temperature and 10°C was assessed using a borate buffer. The barrier sheet of the device was composed of PVC, the carrier material used was EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was cresol red 0.01%, the buffer material used was 0.025 M Borax (pH 9.36) and the outer layer was composed of Saran Wrap. The device was placed above the meat for gas phase detection of the reactant molecules.

A color change was observed with the aged meats after 1 hour at room temperature and after 18 hours at 10°C. The color of the indicator material was noted to change from violet (V) to yellow (Y) as spoilage progressed and acidity increased. Significant changes of color are indicated in bold.

Room Temperature			10°C
Color at	0h	1h	18h
Water	V	V	V
Hamburger	fresh V	V	V
	aged V	V↓↓	Y
Chicken	fresh V	V	V
	aged V	V↓↓	Y
Fish	fresh V	V	V
	aged V	V↓↓	Y

Example 10

In this example, the response of the spoilage indicator device to different concentrations of soda water (Canada Dry) was assessed. The barrier sheet was composed of PVC, the carrier material used was EX-L Kimwipe tissue paper (thickness ~18 microns), the indicator material used was phenol red 0.02%, the buffer material used was 0.025 M lysine (pH 8.42) and the outer layer was composed of Saran Wrap. The device was placed above the soda water for gas phase detection of the reactant molecules. The color of the indicator material was read at 15 minute intervals at room temperature.

The color of the indicator material was noted to change from yellow (Y) to red (R) with decreasing CO₂. * These results illustrate that CO₂ concentration decreases over time, indicating the soda water is going flat and becoming unpalatable.

Soda water dilution	0	1/2	1/5	1/10	1/20	Water
Color	Y	YYR	YR	RY	R	R

Those skilled in the art may appreciate the other advantages and uses of the subject matter disclosed herein. Such other advantages, uses and embodiments of the apparatus and methods described herein are included in the following claims.

What is claimed is:

CLAIMS

1. A method of detecting spoilage of a food product comprising the steps of:

5 placing a spoilage indicator device in fluid contact with a food product, said spoilage indicator device having a barrier sheet which is permeable to uncharged molecules under approximately 200 daltons while being substantially impermeable to charged molecules and larger uncharged molecules, said spoilage indicator device further containing a carrier having a first substantially planar surface and a second substantially planar surface,
10 said carrier having a pH indicator and a buffer material impregnated therein, said pH indicator being capable of producing a detectable change upon reacting with a reactant molecule which can pass through said barrier sheet and which is produced in a food product when the food product is undergoing spoilage, said spoilage indicator device further containing an outer layer, said spoilage indicator device being arranged so that said barrier
15 sheet is in fluid contact with said food product, said barrier sheet separating said first planar surface of said carrier from said food product, said outer layer is in fluid contact with ambient atmosphere or said food product, said outer layer separating said second planar surface of said carrier from said ambient atmosphere or food product;

20 allowing said reactant molecule produced in said food product to traverse said barrier sheet, to contact said carrier and to react with said pH indicator therein; and

observing said spoilage indicator device to determine whether said detectable change has occurred in said carrier, whereby said detectable change indicates a
25 build-up of said reactant molecule in said food product and thereby indicates food spoilage.

2. The method of claim 1, wherein said reactant molecule is carried in a liquid phase and said pH indicator is capable of reacting with said reactant molecule in said liquid phase.
30

3. The method of claim 1, wherein said reactant molecule is carried in a gaseous phase and said pH indicator is capable of reacting with said reactant molecule in said gaseous phase.

35 4. The method of claim 1, wherein said reactant molecule is carried in an acid and said pH indicator is capable of reacting with said reactant molecule in said acid.

5. The method of claim 1, wherein said reactant molecule is carried in a base and said pH indicator is capable of reacting with said reactant molecule in said base.

6. The method of claim 1, wherein said detectable change is a visual detectable
5 change.

7. The method of claim 6, wherein said barrier sheet is composed of a hydrophobic material.

10 8. The method of claim 7, wherein said barrier sheet is selected from the group consisting of polyethylene and polyvinyl chloride.

9. The method of claim 8, wherein said barrier sheet has a thickness in the range of 10 to 15 microns.

15 10. The method of claim 9, wherein said barrier sheet is tightly contiguous to the first planar surface of said carrier.

11. The method of claim 6, wherein said carrier is a paper.

20 12. The method of claim 11, wherein said carrier has a thickness in the range of 15 to 20 microns.

25 13. The method of claim 6, wherein said pH indicator material is selected from the group consisting of phenol red, cresol red and *m*-cresol purple.

14. The method of claim 6, wherein said buffer is effective within the pH 8-10 range.

30 15. The method of claim 14, wherein said buffer is an amino acid.

16. The method of claim 15, wherein said buffer is lysine or glycine.

35 17. The method of claim 6, wherein said outer layer is contiguous to said second planar surface of said carrier.

- 24 -

18. The method of claim 17, wherein said outer layer has a thickness in the range of 5 to 25 microns.

19. The method of claim 18, wherein said outer layer is in contact with the ambient atmosphere and said outer layer consists of a gas-impermeable material.

20. The method of claim 19, wherein said outer layer consists of a gas-impermeable material selected from the group consisting of polyester, cellulose acetate, polyolefin, polypropylene polystyrene and polyvinylidene chloride.

21. The method of claim 18, wherein said outer layer is in contact with the gas phase enveloping said food product and said outer layer consists of a gas-permeable material.

22. The method of claim 21, wherein said outer layer consists of polyvinyl chloride or polyethylene.

23. A spoilage indicator device for placing in fluid contact with a food product to detect spoilage, said spoilage indicator device having a barrier sheet which is permeable to uncharged molecules under approximately 200 daltons while being substantially impermeable to charged molecules and larger uncharged molecules, said barrier sheet being arranged so in use it is in fluid contact with said food product, said spoilage indicator device further containing a carrier having a first substantially planar surface and a second substantially planar surface, said carrier having a pH indicator material and a buffer material impregnated therein, said barrier sheet separating a first planar surface of said carrier from said food product, said spoilage indicator device further containing an outer layer, said outer layer separating a second planar surface of said carrier from the ambient atmosphere or gaseous phase surrounding said food product, said pH indicator being capable of producing a detectable change upon reacting with a reactant molecule which can pass through said barrier sheet and which is produced in said food product when said food product is undergoing spoilage and traverses said barrier sheet.

24. The device of claim 23, wherein said detectable change is a visual detectable change.

25. The device of claim 24, wherein said barrier sheet is composed of a hydrophobic material.

- 25 -

26. The device of claim 25, wherein said barrier sheet is selected from the group consisting of polyethylene and polyvinyl chloride.

5 27. The device of claim 26, wherein said barrier sheet has a thickness in the range of 10 to 15 microns.

28. The device of claim 27, wherein said barrier sheet is contiguous to the first planar surface of said carrier.

10

29. The device of claim 24, wherein said carrier is a paper.

30. The device of claim 29, wherein said carrier has a thickness in the range of 15 to 20 microns.

15

31. The device of claim 23, wherein said outer layer is contiguous to a second planar surface of said carrier.

20 32. The device of claim 23, wherein said outer layer has a thickness in the range of 5 to 25 microns.

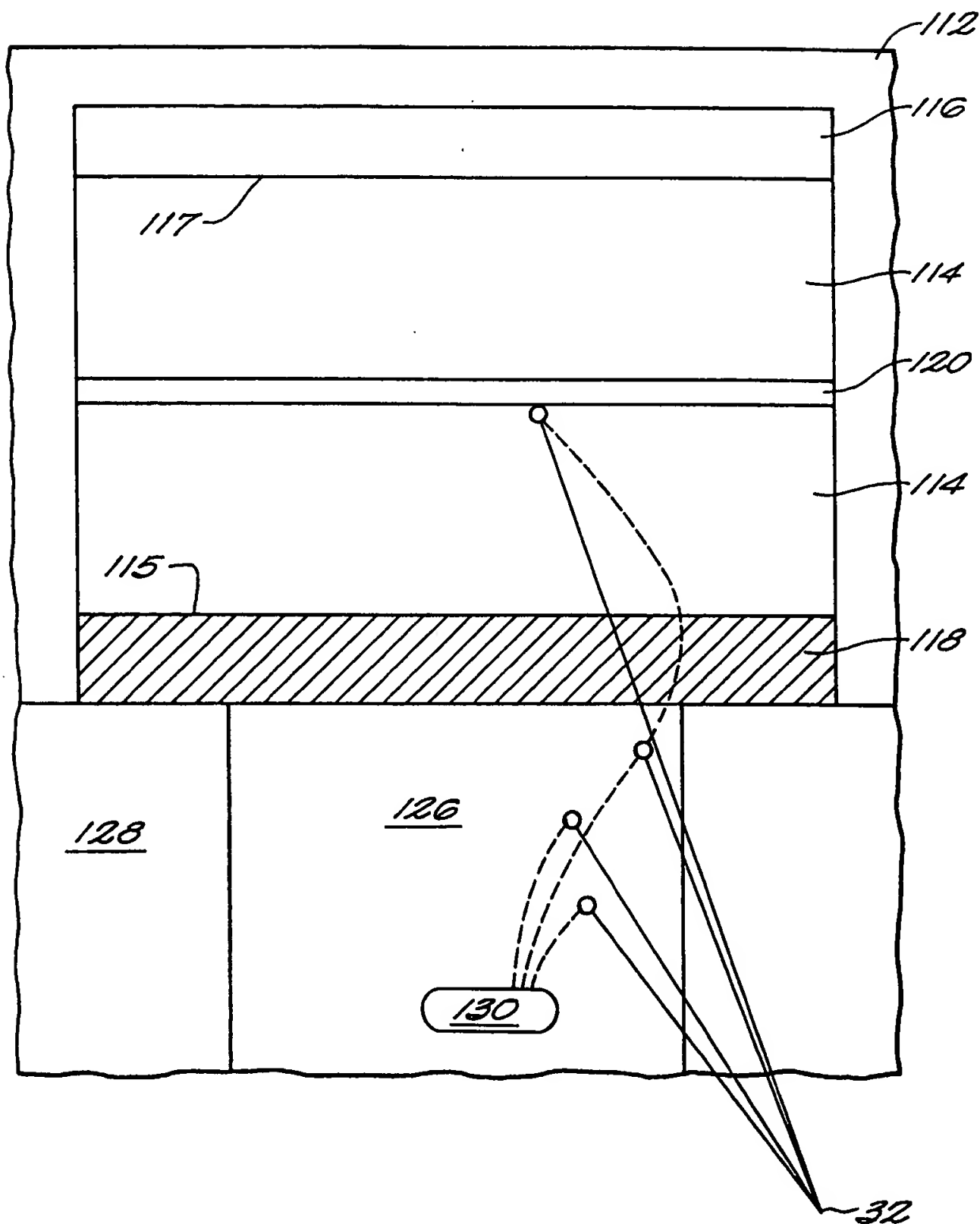
33. The device of claim 32, wherein said outer layer is in contact with the ambient atmosphere and said outer layer consists of a gas-impermeable material.

25 34. The device of claim 33, wherein said outer layer consists of a gas impermeable material selected from the group consisting of polyester, cellulose acetate, polyolefin, polypropylene polystyrene and polyvinylidene chloride.

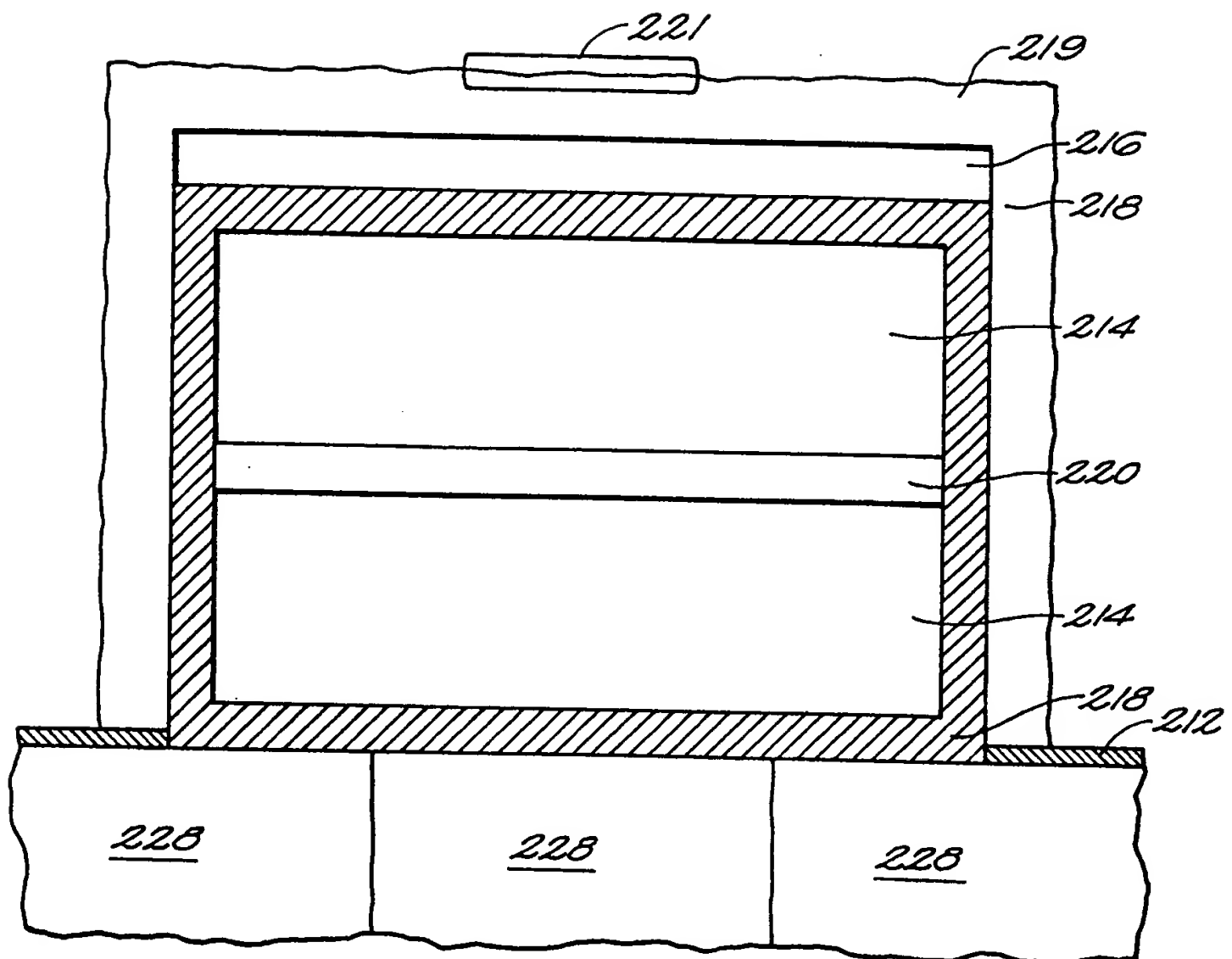
30 35. The device of claim 32, wherein the outer layer is in contact with the gas phase enveloping the food product, the outer layer consists of a gas-permeable film.

36. The device of claim 35, wherein said outer layer consists of polyvinyl chloride or polyethylene.

1/2

**FIG. 1**

2/2

**FIG. 2**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/20097

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 31/00, 31/16, 33/02, 21/00

US CL : 436/2, 20, 164, 163; 422/55, 61

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/2, 20, 164, 163; 422/55, 61

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, WPIDS

search terms: food, meat, spoilage, bacterial, microbial, indicator

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,285, 697 A (NEARY) 25 August 1981, entire document.	1-31
Y	US 5,053,339 A (PATEL) 01 October 1991, column 12, lines 33-63 and column 15, Table 2 and 3.	1-31
Y	VWR Scientific, Co. The VWR Scientific Catalog. 1987/1988, pages 776-777, see entire document.	1-31
A	US 5,306,466 A (GOLDSMITH) 26 April 1994, entire document.	1-36



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

18 DECEMBER 1997

Date of mailing of the international search report

03 FEB 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ELIANE LAZAR-WESLEY

Telephone No. (703) 308-0196